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14. ABSTRACT

The purposes of this study are to provide quantitative estimates of 1) the effective amount of vitamin D produced in the skin as a function of skin pigmentation; and 2) the rate of utilization of vitamin D as a function of ethnicity. The outcome will be estimates of the amount of vitamin D that must be given orally to military personnel of different races and in different assigned locations so as to ensure and maintain normal vitamin D status. In the first 39 months' work (the period covered by this report), we have accumulated 80+% of the targeted specimens for both objectives, in a racially diverse sample. In addition we have augmented our findings from naturally sun-exposed individuals to include responses in volunteers receiving controlled doses of UV-B. Analyses are continuing and will be completed within the coming months. No final quantitative results will be available until all the measurements have been made and analyzed as a unit.

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INTRODUCTION

The purpose of this project is to develop quantitative estimates of 1) the amount of vitamin D produced by skin exposure to sunlight (Experiment 2, below), and 2) the amount of oral vitamin D that must be given to supplement solar inputs so as to achieve desired vitamin D status in military personnel of differing races and skin pigmentation (Experiment 1, below).

This is the fifth annual report with respect to the above-referenced award. Although the award was made as of 1 October 2001, authorization to proceed was not received from USAMRMC until 15 July 2002. Hence this report, although technically covering the first five years of the award, describes work performed only from 15 July 2002 until submission of this report, i.e., a period of little more than four years. This is anticipated to be the last of the annual reports. Next year's is scheduled to be the final report on the entire project.

BODY OF REPORT

Work Performed: Experiment 1. The purpose of Experiment 1 was to quantify the ethnic differences (if any) in metabolism of known inputs of vitamin D_3. It is designed to be executed over the winter months when solar vitamin D input is minimal and total input can be controlled by the investigators through daily oral dosing of controlled quantities of vitamin D_3. Our plan was to split the project into two phases, studying doses of zero and 1000 IU/d during the first year of the project, and doses of 5,000 and 10,000 IU/d during the second year. We have completed both the specimen acquisition and the biochemical analyses for this experiment, but beyond a preliminary analysis which indicates that African American metabolism of vitamin D is similar to that of Caucasians, we have not yet fully analyzed the patterns produced by the data.

Racial and sex distribution of participants in Experiment 1 is as follows:

	Non-Hispanic Caucasian	Hispanic	African-American	Totals
Male	5	16	3	24
Female	13	9	48	70
Totals	18	25	51	94

These numbers are above our target figure of 80 enrollees.

As this phase of the project had been completed prior to last year's progress report, no further work was done with respect to specimen acquisition or participant interaction in the past 12 months. Final data analysis is deferred until all data have been compiled.

Work Performed to Date: Experiment 2. The purpose of Experiment 2 is to quantify the serum 25(OH)D response (and its physiological correlates) to summer sun exposure in persons with a wide range of skin pigmentation. As of 30 September 2005 we had enrolled 79 individuals and had obtained both the first (i.e., late summer) and the second (late winter) measurements for most of them, as specified for Experiment 2. This number (79) was just shy of our target of 80 participants, with the shortfall being because of the loss of a project manager in two successive

years at the peak of late summer recruitment. In this past year we enrolled 10 additional participants and have three more who are tentatively scheduled to enter by October 1. The racial and sex breakdown of the group so far recruited into Experiment 2 is as follows:

	Non-Hispanic Caucasian	Hispanic	African-American	Totals
Male	19	6	31	56
Female	13	2	18	33
Totals	32	8	49	89

For each of these subjects we have obtained (or are in the process of obtaining) the suite of specimens/measurements specified in the approved protocol, i.e., history of sun exposure by duration and clothing type; skin pigmentation by reflectance meter measurement; calcium absorption efficiency; measurements of the full set of hormones regulating the calcium economy [i.e., PTH, $1,25(OH)_2D_3$, 25(OH)D], as well as blood vitamin D levels themselves, urine calcium excretion, and bone densitometry. While degree of sun exposure varied, all participants were selected because they self-reported substantial mid-day sun exposure throughout most or all of the summer.

As noted previously, we have sufficient data to permit several observations. Many of them (e.g., black-white differences) were already known from other studies; our goal in this project was to quantify them so as to develop better estimates of the amount of vitamin D producible in the skin in persons of varying color. This will allow development of evidence-based guidelines for vitamin D supplementation of DoD personnel. Findings to date are as follows:

- African-Americans have lower serum 25(OH)D values than whites at both summer and winter measuring points. Although we have too few Hispanic subjects for a precise estimate, their data tend to be intermediate between blacks and whites.
- African-Americans, working outdoors, elevate their serum 25(OH)D levels to an extent not clearly different from whites (based on data available to date).
- Neither serum calcium nor urine calcium excretion differs between the late summer and late winter measurement times. Hence there is no evidence of hyperabsorption of calcium.
- Serum PTH rises significantly from late summer to late winter, indicating that the late winter level of 25(OH)D is physiologically inadequate (thus evoking increased PTH secretion).
- Calcium absorption efficiency is slightly (but significantly) higher at the late summer measurement point. This finding, as with the PTH difference, indicates that, by late winter, there is not only chemical evidence of vitamin D deficiency (i.e., low serum 25(OH)D), but physiological evidence as well (i.e., lower intestinal calcium absorption). Thus, steps to correct this inadequacy are likely to produce a benefit.

The recruitment problem we have had for this component of the project is partly a reflection of national experience in recruiting persons of color into any clinical research study, and partly due to the fact that the outdoor workers who are our target population could not easily afford losing a

day's work (and pay). To compensate for this recruitment shortfall, we have devised a different approach (described last year) which expands the pool of subjects and also provides a more precise quantification of UV-B exposure.

This revised strategy uses a dermatologist's UV light box (National Biological HOUVA II UV Light Booth, National Biological Corp., Twinsburg, OH), delivering calibrated whole body exposure to selected UV-B light intensities, measured in mJoules. (For reference purposes, 15 minutes of outdoor sun exposure at our latitude in July at mid day delivers ~20 mJ.) Volunteers receive 3× weekly radiation at different doses. Serum 25(OH)D level is followed for 8 weeks, and is analyzed as a function of constitutive skin tone. Skin tone, described in previous reports, is measured using an IMS Smart-Probe 400 (IMS, Inc., Milford, CT), which distinguishes the three principal components of skin color: black (melanin), yellow (carotene, etc.), and red (blood flow). The melanin component absorbs UV-B and reduces its ability to make vitamin D. "Constitutive" skin color is given by the melanin level on typically unexposed skin (e.g., the inside surface of the upper arm).

As would be predicted, 25(OH)D response to UV-B exposure is inversely proportional to constitutive skin color. We reported last year that serum 25(OH)D response was a direct function of both dose (in mJ) and constituitive skin color (i.e., skin "lightness"), measured at that time in 48 subjects. (This number is in addition to the outdoor summer workers described above.) We now have such measurements on 73 individuals (with one still in process), with greater emphasis on individuals with low lightness scores, i.e., persons of substantial color. Preliminary analysis of these data suggest that, while skin color is, as expected, a factor in 25(OH)D response, dose is not (or at least only weakly so). This had not been anticipated and its implications are being explored. One possible explanation is a limited concentration of 7-dehydrocholesterol in the skin of highly pigmented individuals. 7-dehydrocholesterol is the immediate precursor of pre-vitamin D and is the compound that is acted upon by UV radiation. Reduced skin 7-dehydrocholesterol has been previously reported with aging and in individuals with severe chronic kidney disease. So it is not an altogether implausible explanation for a blunted response to UV-B radiation in persons of color. If this turns out to be the case, then it follows that vitamin D adequacy in military personnel will depend almost totally on periodic oral supplementation.

Our purpose in this aspect of the project was not to find what was already known (i.e., response is a function of skin color), but to quantify it – i.e., to define how much sun exposure is needed to produce desired 25(OH)D values, and to do so as a function of inherent skin color. Results, when combined with the findings of Experiment 1, will translate to the oral dosing of vitamin D needed to achieve the desired serum 25(OH)D values irrespective of race or color.

Work Plan for the Forthcoming Year. We have enrolled 10 new participants in Experiment 2 (the outdoor worker, summer sun exposure phase of the project), since last years's report, with three more in prospect. They will complete their participation in February 2007, at which time all participant interaction and specimen acquisition will have been completed. In addition, we will complete the subjects now underway in the controlled UV-B exposure phase of the project. This work takes us well above our original enrollment targets, but is considered necessary since detailed questioning of several of the previously enrolled outdoor workers revealed little actual sun exposure for several of them. We anticipate spending most of the coming year on this additional work and on completion of analysis of acquired specimens, on data clean-up, specimen reanalysis (as needed), statistical modeling and analysis, and report generation. The

rate of expenditure the past two years has been scaled back to match the slower than anticipated recruitment.

KEY RESEARCH FINDINGS

Key research findings (on the still incomplete dataset) are as set forth under Experiments 1 and 2, above. In addition to the presentation described in prior years' progress reports, a portion of these results were presented in poster form at the meeting of the American Society for Bone and Mineral Research in Philadelphia, PA, September 26, 2006. Copies of the Abstracts are attached as Appendix I. Full analysis and publication must wait completion of analyses for all the subjects during this coming year.

REPORTABLE OUTCOMES

As noted in the foregoing, the reportable outcomes from this study will consist of 1) best quantitative estimates of skin production of vitamin D as a function of skin pigmentation and extent of skin exposure; and 2) best quantitative estimates of rate of utilization of vitamin D_3 as a function of race/ethnicity. Taken together, both will yield estimates of the quantity of vitamin D that must be given to military personnel to ensure maintenance of desired vitamin D status. Since much of the work is still underway, final quantitative estimates are not yet available. However, from the data produced so far, it seems safe to say that we will be able to produce the projected quantitative estimates. Moreover, secondary findings will become available and doubtless further such will develop as we accumulate more measurements. An example of such secondary data can be found in the Abstracts attached in the Appendix.

CONCLUSIONS

None to date except as described above from partial analysis of the sample.

REFERENCES

1. Armas LAG, Heaney RP, Barger-Lux MJ, Huerter C, Lund R. The effects of UV-B light on serum 25(OH)D in humans. *J Bone Miner Res* 20 (Suppl 1):S188, 2005.
2. Armas LAG, Heaney RP, Barger-Lux MJ, Huerter C, Lund R. The effects of UV-B light on serum 25(OH)D in humans with dark skin tones. *J Bone Miner Res* 21 (Suppl 1):S449, 2006.

APPENDIX

Reference 1

The Effects of UV-B Light on Serum 25(OH)D in Humans L. A.G. Armas, R. P. Heaney, M.J. Barger-Lux, C. Huerter, R. Lund, Creighton University, Omaha, Nebraska

We report results of work to quantify the relationship of skin color and 25(OH)D response to graded doses of UV-B light delivered by a light booth. The subjects (n=48, age 21-49 yr, females = 28, males = 20) were healthy indoor workers with limited non-solar sources of Vitamin D. They were divided into 5 treatment groups based on their self-reported susceptibility to tan or burn (Fitzpatrick skin types I-VI).

Data were gathered from January through April. We determined BMI, 25(OH)D, Ca^{2+} and PTH at baseline. We used a portable colorimeter that utilizes the CIE L*a*b* color system to measure constitutive skin color of the upper inner arm and facultative skin color of the forearm. The subjects were exposed to UV-B light from a UV light booth 3 times a week for 4 weeks (12 treatments) in graded doses ranging from 40mJ to 80mJ per treatment. 25(OH)D was drawn weekly during and 4 weeks after completion of UV-B treatment.

There was a rise in 25(OH)D of 29.9 nmol/L(median; interquartile range 24.3-40.7) during the 4 weeks of UV-B treatment and a fall 4 weeks after UV-B treatment ceased of 3-14% from peak 25(OH)D levels. The "L*" values of exposed skin did not vary significantly throughout the treatment period. There was a significant correlation between "L*" readings (a continuous darker-to-lighter scale) of baseline facultative and constitutive skin color and 25(OH)D response per mJ of UV-B light given (r^2=0.535, r^2=0.551)(i.e. the lighter skinned subjects had a greater response).

In conclusion, increase in 25(OH)D per mJ of UV-B exposure was related to the "L*" value of skin at baseline. This increase in 25(OH)D was achieved without changing "L*" values (i.e. becoming darker).

Reference 2

The Effects of UV-B Light on Serum 25(OH)D in Humans with Dark Skin Tones. L. A.G. Armas, R. P. Heaney, M.J. Barger-Lux, C. Huerter, R. Lund, Creighton University, Omaha, Nebraska

We report results of work to quantify the relationship between dark skin tone and 25(OH)D response to graded doses of UV-B light delivered by a light booth. The subjects (n=23, age 20-48 yr, females = 10, males =13) were healthy indoor workers with limited non-solar sources of Vitamin D. They had Fitzpatrick skin types V & VI based on their self-reported susceptibility to tan or burn.

Data were gathered from January through May for 2 consecutive years. We determined BMI, 25(OH)D, Ca^{2+} and PTH at baseline. We used a portable colorimeter that utilizes the CIE L*a*b* color system to measure constitutive skin tone of the upper inner arm. The subjects had near whole body exposure to UV-B light from a UV light booth 3 times a week for 4 weeks (12 treatments) in graded doses ranging from 20mJ to 80mJ per treatment. We estimate that about 90% of the skin was exposed to UVB light. Serum 25(OH)D was drawn each week during and 4 weeks after completion of UV-B treatment.

The subjects had an L* score less than 60 on the dark to light scale. Their median L* score was 50; interquartile range 47-53. The increase in 25(OH)D was .0383 nmol/L per mJ of UVB light received (median; interquartile range 0.0290-0.0494). Using data from a previous study we calculate that one IU of cholecalciferol increases 25(OH)D by .0021 nmol/L. We used data collected from the USDA UV-B Monitoring and Research Program to find the amount of UV-B light that is received in July at noon in Nebraska (41.2° N latitude). We found by simple algebraic method that 66 minutes of July noon sunlight will provide 90 mJ of UVB light. Using the data we collected on dark skin types this would provide a rise of 25(OH)D equivalent to an oral dose of 800 IU of cholecalciferol if 45% of the skin is exposed (wearing a short sleeved shirt and shorts).

In conclusion, dark skin types require about an hour of intense sunlight daily to provide the equivalence of 800 IU of oral cholecalciferol.